Calculation of Relative Solvation Free Energy Differences by Thermodynamic Perturbation Method: Dependence of Free Energy Results on Simulation Length

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ABSTRACT: Molecular dynamics (MD) simulations in conjunction with the thermodynamic cycle perturbation approach has been used to calculate relative solvation free energies for acetone to acetaldehyde, acetone to pyruvic acid, acetone to 1,1,1-trifluoroacetone, acetone to 1,1,1-trichloroacetone, acetone to 2,3-butanedione, acetone to cyclopropanone, and formaldehyde hydrate to formaldehyde. To evaluate the dependence of relative solvation free energy convergence on MD simulation length and starting configuration two studies were performed. In the first study, each simulation started from the same well-equilibrated configuration and the length was varied from 153 to 1530 ps. In the second study, the relative solvation free energy differences were calculated starting from three different configurations and using 510 ps of MD simulation for each mutation. These results clearly indicate that, even for molecules with limited conformational flexibility, a simulation length of 510 ps or greater is required to obtain satisfactory convergence and, for the mutations of large structural changes between reactant and product, such as cyclopropanone to acetone, require much longer simulation lengths to achieve satisfactory convergence. These results also show that performing one long simulation is

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better than averaging results from three shortest simulations of the same length using different starting conformations. © 1999 John Wiley & Sons, Inc. J Comput Chem 20: 1018–1027, 1999

Keywords: thermodynamic cyclic perturbation approach; solvation free energies; convergence; molecular dynamics simulations; AMBER force field; thread method

Introduction

Since early 1980, over 100 studies have been published in the literature reporting the calculation of relative solvation (with explicit solvent) free energies between two similar molecules using molecular dynamics (MD) or Monte Carlo (MC) simulations in conjunction with the thermodynamic cycle perturbation approach.[1] It is clear from previous reports that MD simulations using explicit solvent yield free energies that are not fully converged, due to limited computer resources. Convergence is therefore a potential problem for calculations of this type. Nevertheless, the methodology has been successful in obtaining satisfactory agreement with experimental results within ± 1.0 kcal/mol. The work of McCammon[2a] and Kollman[2b] examined the behavior of the calculated free energy with the length of the simulation, and concluded that simulation times significantly longer than those typically used (> 200 ps) may be required to yield reliable and accurate results. In addition, others[2c–f] have studied the convergence problems in the simulations—in particular, Chipot et al.,[2f] who investigated the convergence behavior of potential of mean force (PMF) calculations using free energy perturbation (FEP), thermodynamic integration (TI), and slow growth (SG) techniques, and concluded that proper convergence of the free energy calculations requires simulation times much longer than previously estimated.

The purpose of this study was twofold. First, our recent calculations[3] for the relative hydration free energies between two similar molecules included contributions from both quantum mechanical gas-phase free energies and solvation free energies. Because the relative solvation free energy difference of differences ($\Delta\Delta\Delta G_{sol}$) between two products and two reactants is small, large errors in these calculations will have a significant effect on the calculated relative hydration free energies. Therefore, it was important to obtain good convergence in the solvation free energies to reduce errors in the relative hydration free energies. Second, we wanted to assess the convergence of solvation free energy results as a function of MD simulation length and starting configurations. The good agreement between calculated relative hydration free energies[3] and experimental results reported for all these systems have indicated that the calculated relative solvation free energies are accurate, even though experimental solvation free energies are not available for several of the systems considered in this study.

Reported herein are studies that evaluate the effect of the length of simulation on free energy. In the first study, each simulation started from the same well-equilibrated configuration and the length was varied from 153 to 1530 ps. In the second study, the relative solvation free energy difference was calculated starting from three different configurations, using 510 ps of MD simulation for each mutation. Although the molecules considered (Fig. 1) for these studies were small, the molecules selected were based on their interest in the calculation of relative hydration free energies[3a]. In each study, seven mutations were evaluated: (1) acetaldehyde to acetone; (2) pyruvic acid to acetone; (3) 1,1,1-trifluoroacetone to acetone; (4) 1,1,1-trichloroacetone to acetone; (5) 2,3-butanedione to acetone; (6) cyclopropanone to acetone; and (7) formaldehyde hydrate to formaldehyde.

1. Acetaldeyde = CH_3COH
2. Acetone = CH_3COCH_3
3. Pyruvic Acid = $CH_3COCOOH$
4. 1,1,1-Trifluoroacetone = CF_3COCH_3
5. 1,1,1-Trichloroacetone = CCl_3COCH_3
6. 2,3-Butanedione = $CH_3COCOCH_3$
7. Cyclopropanone = 
8. Formaldehyde = $HC(O)H$
9. Formaldehyde hydrate = $HC(OH)_2H$

FIGURE 1. Compounds studied.

Simulation Methods

Solvation is a complex physical process that is usually difficult to simulate. Because free energy is a state function, nonphysical but computationally more suitable processes can be used to form a thermodynamic cycle. Figure 2 illustrates such processes for the calculation of solvation free energy. The calculated relative change in the solvation free energy is as follows:

$$\Delta\Delta G_{sol} = \Delta G_{aq} - \Delta G_{gas} = \Delta G_2 - \Delta G_1 \qquad (1)$$

The free energy changes ΔG_{gas} and ΔG_{aq} are computed independently by transforming the Hamiltonian of a reactant state (R) into a product (P) state during the molecular dynamics simulations in the appropriate phase. A perturbation approach that accomplishes this transformation is:

$$H(\lambda) = H_0 + H_1(\lambda); \qquad \lambda = 1 \text{ to } 0 \qquad (2)$$

where H_0 is the Hamiltonian of an unperturbed system, which consists of reactant(s) and the environment. $H_1(\lambda)$ is a perturbation such that $H_1(0) = 0$ and $H_0 + H_1(\lambda)$ is the Hamiltonian of a system that consists of a product and its environment. The transformation is carried out in small increments of $\Delta\lambda$. The free energy difference between states is defined at λ and $\lambda + \Delta\lambda$

$$G(\lambda+\Delta\lambda) - G(\lambda) = -K_B T \ln\langle\langle \exp(-(H(\lambda+\Delta\lambda) - H(\lambda))/K_B T)\rangle\rangle\lambda \qquad (3)$$

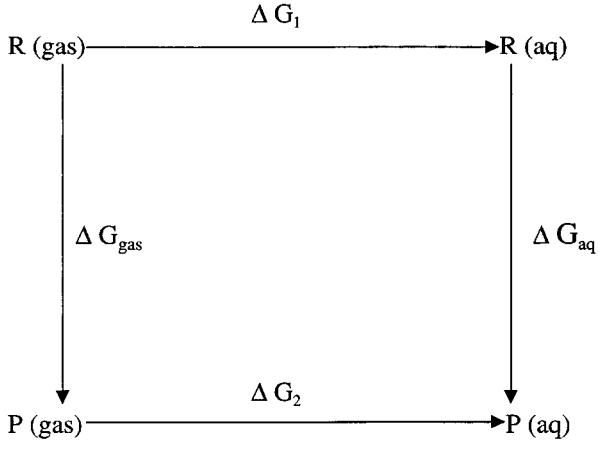

FIGURE 2. Cycle used for calculating relative solvation free energy differences.

where K_B is Boltzmann's constant, T is the absolute temperature, and the ensemble average ($\langle\langle \ldots \rangle\rangle$) is computed as a time average over a molecular dynamics trajectory with an evolution that is governed by the Hamiltonian $H(\lambda)$. The total free energy change from the initial state to the final state is computed by the summation of these incremental free energy changes in each window between $\lambda = 0$ to $\lambda = 1$.

Computational Details

The AMBER[4] all-atom force field was used. For standard atoms, van der Waals parameters and bond, angle, and torsional force constants were taken from the AMBER database.[4] For nonstandard atoms, force field parameters were estimated from parameters available in the AMBER database for similar species. For the solute atoms, atomic partial charges were obtained by fitting with the CHELPG[5] to the quantum-mechanical electrostatic potential computed from the *ab initio* 6-31G** wave functions calculated with GAUSSIAN-92. All equilibrium bond lengths, bond angles, and dihedral angles were taken from *ab initio*-optimized geometries at the 6-31G** basis set level. Torsion parameters for gem-diols were derived from torsion energy barrier profiles of several representative hydrates at the 6-31G** basis set level.[3a] Torsion energy parameters were adjusted in the AMBER force field such that molecular mechanics calculations reproduced quantum-mechanical torsion energy barrier profiles qualitatively. For all compounds, equilibrium geometries, atomic partial charges, and nonstandard force field parameters not available in the AMBER force field have been submitted as supplementary material (Table IV).

The SPC / E water[7] model potential was used in the solvation calculations. Initially, each solute was immersed in a large box of equilibrated SPC/E water[7b] and waters overlapped by solute atoms were removed. The box was further truncated to yield an 11-Å rectangular box of water molecules surrounding the solute atoms. Relative changes in the solvation free energies were calculated using molecular dynamics simulations in conjunction with the thermodynamics perturbation approach. The window method implemented in the AMBER program was used. Molecular dynamics simulations were carried out using periodic boundary conditions in all directions. Newton's equations of motion for all the atoms were solved using the

Verlet algorithm with a 2-fs time step. SHAKE was used to constrain all bond lengths. Constant temperature (N, P, T ensemble) was maintained by scaling velocities of all atoms in the system. The nonbonded interaction energies were calculated using an 11-Å residue-based cutoff. Relevant computational details for all the systems are given in the Table I.

The ''thread''[8] method was used to mutate cyclopropane to acetone (molecule 6, Fig. 1) due to their significant structure dissimilarity. For this mutation, reactant (cyclopropane) and product (acetone) are threaded together at carbonyl carbon position. Accordingly, a single topology is defined for atoms that are identical in both molecules (carbonyl carbon and oxygen), which means that the force constants and equilibrium geometries are the same (partial charges can vary). The remaining portions of each molecule undergo the transformation. The starting (reactant) and ending (product) topologies are defined with the appropriate geometries: one beginning and the other ending the simulation entirely as dummy atoms. Dummy atoms are identical to real atoms except that the Lennard–Jones parameters and atomic charges are set to zero. At intermediate points during the transformation, all atoms, in both topologies, have fractional Lennard–Jones parameters and charges. Molecules with both topologies interact with the environment, but not with each other. As with the solvation free energy calculation of the water molecule, a two-stage procedure was used to obtain better convergence.[8] During the first stage, the charges of the reactant atoms were slowly decreased to zero while the Lennard–Jones parameters of the product atoms were slowly increased to the appropriate values. During the second stage, the Lennard–Jones parameters of the reactant atoms were slowly decreased to zero while the charges of the product atoms were slowly increased to the appropriate values. Each stage of the simulation was performed using 51 windows. Initially, the system was minimized and equilibrated for 20 ps, which was followed by several independent molecular dynamics simulations to assess the convergence of the calculated results.

For all other simple mutations (Fig. 1), a single topology was used to accomplish the transformation from one molecule to a related molecule. In this commonly used method, appropriate reactant atoms are transformed to the corresponding product atoms. Here, 51 windows were used for each mutation. Initially, each system was minimized and equilibrated for 20 ps, which was followed by several independent molecular dynamics simulations for each system in order to assess the convergence of the calculated results. For all relative solvation free energy calculations, the errors were estimated for each window by dividing the window statistics into four groups and computing the standard deviation. The root mean square of these window errors is reported as a measure of the statistical uncertainty in the results for each complete mutation.

Results and Discussion

To assess convergence of the solvation free energy calculations and its dependence on MD simulation length, seven sets of molecules were evaluated using simulation lengths that ranged from

TABLE I.
Computational Details for FEP Calculations.

Serial Number	System	Box Size[a]	Number of Water Molecules	Nonbonded Cutoff Distance
1	CH_3COCH_3 — $HCOCH_3$	26.5 × 24.8 × 24.0	520	11.0 Å
2	CH_3COCH_3 — $CH_3COCOOH$	27.2 × 25.2 × 23.7	531	11.0 Å
3	CH_3COCH_3 — CF_3COCH_3	26.7 × 24.9 × 24.0	523	11.0 Å
4	CH_3COCH_3 — CCL_3COCH_3	26.8 × 25.2 × 24.5	544	11.0 Å
5	CH_3COCH_3 — $CH_3COCOCH_3$	27.1 × 25.3 × 23.6	530	11.0 Å
6	CH_3COCH_3 — cyclopropanone	26.6 × 25.0 × 24.1	525	11.0 Å
7	HCOH — $HC(OH)_2H$	24.7 × 24.2 × 23.6	466	11.0 Å

[a]Units are in cubic angstroms.

153 to 1530 ps, starting from the same well-equilibrated configurations. The calculated results for each mutation are given in Table II. The computed relative solvation free energy difference between acetaldehyde and acetone were -0.56 ± 0.37 kcal/mol (150 ps), -0.52 ± 0.29 kcal/mol (306 ps), -0.44 ± 0.22 kcal/mol (510 ps), -0.45 ± 0.17 kcal/mol (714 ps), and -0.40 ± 0.11 kcal/mol (1530 ps). Although shorter simulation lengths had increased variability, the results indicate that MD simulation lengths of 510 ps or longer gave good agreement with the experimental value of -0.3 kcal/mol. As expected, the standard deviation of the calculated results went down as molecular dynamics simulation length increased from 153 ps (± 0.37) to 1530 ps (± 0.11). Furthermore, a plot of the calculated relative solvation free energy difference for acetaldehyde to acetone as a function of λ (Fig. 3) shows that, at the MD length of 153 ps, there was a wide variation in the relative solvation

TABLE II.
Relative Solvation Free Energy Results.[a]

System	Length of MC Run (ps)	$\Delta\Delta G^{Cal}$	$\Delta\Delta G^{Expt}$
CH_3COCH_3 — $HCOCH_3$[b]	153	-0.56 ± 0.37	-0.3
	306	-0.52 ± 0.29	
	510	-0.44 ± 0.22	
	714	-0.45 ± 0.17	
	1530	-0.40 ± 0.11	
CH_3COCH_3 — $CH_3COCOOH$[b]	153	10.31 ± 1.04	
	306	10.09 ± 0.82	
	510	10.01 ± 0.72	
	714	9.96 ± 0.56	
	1530	9.84 ± 0.48	
CH_3COCH_3 — CF_3COCH_3[b]	153	0.69 ± 0.50	
	306	0.54 ± 0.39	
	510	0.58 ± 0.25	
	714	0.60 ± 0.18	
	1530	0.63 ± 0.13	
CH_3COCH_3 — CCl_3COCH_3[b]	153	3.95 ± 0.59	
	306	3.90 ± 0.46	
	510	3.79 ± 0.33	
	714	3.75 ± 0.26	
	1530	3.67 ± 0.19	
CH_3COCH_3 — $CH_3COCOCH_3$[b]	153	4.05 ± 0.84	
	306	4.24 ± 0.68	
	510	3.78 ± 0.57	
	714	3.63 ± 0.44	
	1530	3.57 ± 0.36	
CH_3COCH_3 — cyclopropanone[b]	153	-0.91 ± 0.70	
	306	-0.79 ± 0.61	
	510	-0.68 ± 0.51	
	714	-0.51 ± 0.36	
	1530	-0.46 ± 0.20	
$HCOH$ — $HC(OH)_2H$[b]	153	8.10 ± 0.97	
	306	7.81 ± 0.82	
	510	7.46 ± 0.67	
	714	7.30 ± 0.56	
	1530	7.20 ± 0.40	

[a] Units are in kilocalories per mole.
[b] Each simulation started from the same well-equilibrated configurations.

free energy as reactant changed to product. This is consistent with the associated larger standard deviation.

In the subsequent study, the relative solvation free energy difference between acetaldehyde and acetone was calculated starting from three different configurations, using 510 ps of MD simulation for each mutation. These results are plotted in Figure 4 as a function of relative solvation free energy vs. λ. The solvation free energies ranged from -0.44 ± 0.22 kcal/mol, -0.42 ± 0.21 kcal/mol, and -0.46 ± 0.23 kcal/mol, for three different simulations, which were in good agreement with the experimental results (-0.3 kcal/mol).[11] These studies show that 510 ps of MD simulation achieved satisfactory convergence. Because the calculated and experimental values were similar, the parameters, the length of the MD simulation, and the methodology appeared satisfactory.

Similar to the acetaldehyde to acetone study, using simulation lengths that ranged from 153 to 1530 ps, starting from the same well-equilibrated configurations, six other mutations were evaluated: pyruvic acid to acetone; 1,1,1-trifluoroacetone to acetone; 1,1,1-trichloroacetone to acetone; 2,3-butanedione to acetone; cyclopropanone to acetone; and formaldehyde hydrate to formaldehyde. The calculated relative solvation free energy results are given in Table II, and also plotted in Figures 5–9 as a function of relative solvation free energy vs. λ (Figs. 6–9 are available in the supplementary material). These results, again, clearly show that standard deviation goes down as MD simulation length increased from 153 (10.31 ± 1.0) to 1530 ps (9.84 ± 0.50) for pyruvic acid to acetone, 153 (0.69 ± 0.5) to 1530 ps (0.63 ± 0.13) for 1,1,1-trifluroacetone to acetone, 153 (3.95 ± 0.59) to 1530 ps (3.67 ± 0.19) for 1,1,1-trichlroacetone to acetone, 153 (4.05 ± 0.84) to 1530 ps (3.57 ± 0.36) for 2,3-

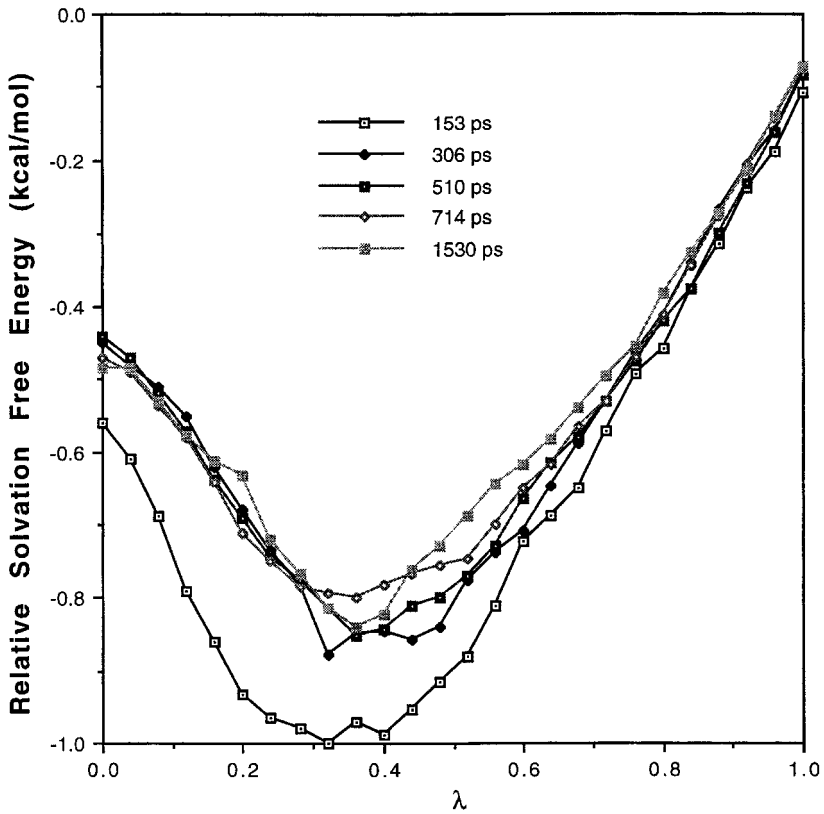

FIGURE 3. Plot of accumulated relative solvation free energy change as a function of λ for the transformation of acetaldehyde ($\lambda = 1$) to acetone ($\lambda = 0$). These calculated results were obtained using different MD simulation lengths (153, 306, 510, 714, and 1530 ps) and same starting configurations.

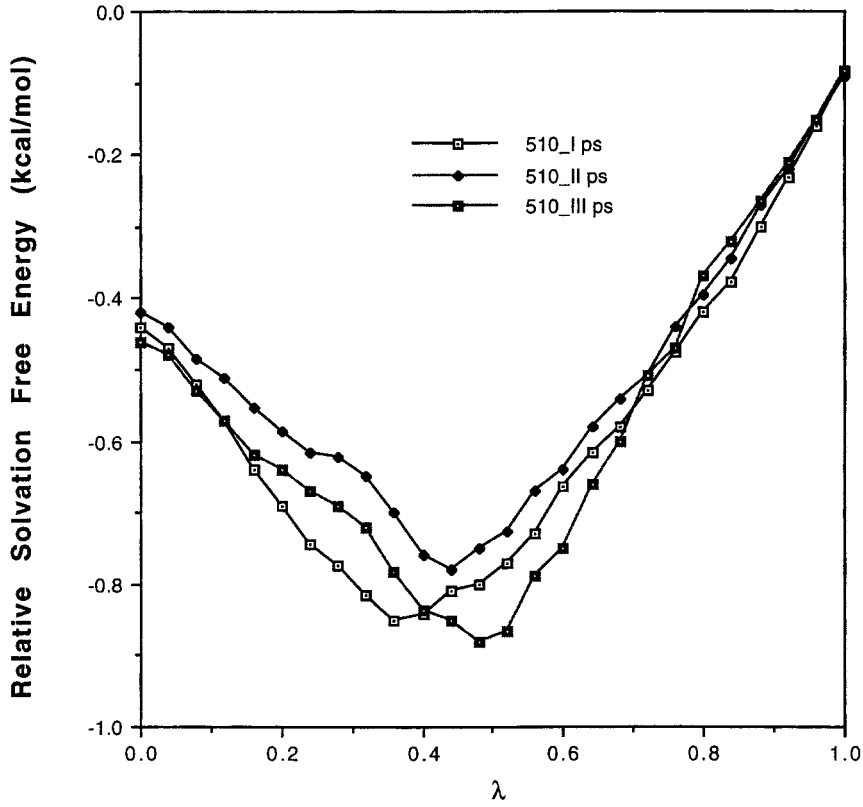

FIGURE 4. Plot of accumulated relative solvation free energy change as a function of λ for the transformation of acetaldehyde ($\lambda = 1$) to acetone ($\lambda = 0$). These calculated results were obtained using a total simulation length of 510 ps and three different starting configurations.

butanedione to acetone, 153 (-0.91 ± 0.70) to 1530 ps (-0.46 ± 0.20) for cyclopropanone to acetone, and 153 (8.1 ± 0.97) to 1530 ps (7.2 ± 0.40) for formaldehyde hydrate to formaldehyde. However, the calculated relative solvation free energies for these systems did not change significantly when the molecular dynamics simulations were longer than 510 ps, even though slightly less satisfactory convergence was observed when the size of the mutation was larger and the flexibility of the molecule increased (e.g., pyruvic acid and 2,3-butanedione). One exception was the mutation between cyclopropanone to acetone where the results indicate unsatisfactory convergence after a 510-ps simulation, because the change involved in the mutation was relatively large (cyclic to acyclic). The mutations of this type require simulation lengths of much longer than 510 ps to achieve satisfactory convergence in the free energy results.

The relative solvation free energy differences for these six systems were also calculated starting from three different configurations and using 510 ps of MD simulation for each mutation; these are given in Table III. The results clearly indicate that the calculated relative solvation free energies did not change significantly, depending upon the starting configurations in the MD simulations. Furthermore, the results indicate that MD simulation length of ≥ 510 ps is required to obtain satisfactory convergence, even for less flexible molecules like those considered in this study. These calculated results indicate that solvation free energies obtained using MD simulation lengths of 153 and 306 ps have large standard deviations, presumably due to poor convergence. However, the results obtained using MD simulation lengths of ≥ 510 ps showed smaller standard deviations and the results were similar for all systems, except for the mutation of cyclopropanone to acetone. Accordingly, MD simulations with explicit solvent lengths of ≥ 510 ps appeared to sample adequately all microstates for all molecules under study (except

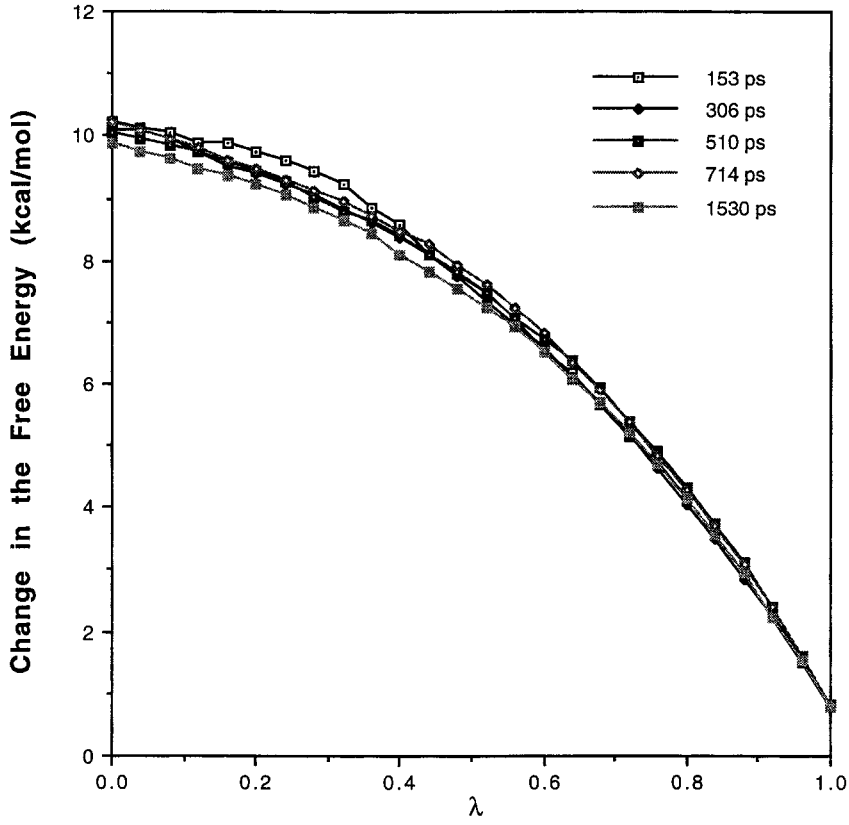

FIGURE 5. Plot of accumulated relative solvation free energy change as a function of λ for the transformation of pyruvic acid ($\lambda = 1$) to acetone ($\lambda = 0$). These calculated results were obtained using different MD simulation lengths (153, 306, 510, 714, and 1530 ps) and the same starting configurations.

cyclopropanone to acetone). To our knowledge, 1530 ps represents the longest MD simulation ever reported with explicit solvent.

Conclusions

Two studies were performed, using seven different systems, in order to evaluate the convergence of relative solvation free energies and its dependence on MD simulation length and starting configurations. In the first study, the relative solvation free energy differences of each system were calculated by varying the molecular dynamics simulation length from 153 ps to 1530 ps. In the second study, the relative solvation free energy differences were calculated starting from three different configurations, using 510 ps of MD simulation for each mutation. These results clearly indicate that, even for the less flexible molecules considered herein, the MD simulation length of $\geq$ 510 ps is required to obtain satisfactory convergence. Accordingly, more flexible molecules are expected to require longer simulation lengths ($>$ 510 ps) to obtain reasonable convergence. Similarly, mutations comprising large structural changes between reactant and product, such as cyclopropanone to acetone, require much longer simulation lengths ($>>$ 510 ps) to achieve satisfactory convergence in the free energy results. Based on the calculated standard deviations for different lengths of MD simulations, these results clearly show that performing one long simulation is better than averaging results from three different simulations of the same length with different starting conformations.

Acknowledgments

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TABLE III.
Relative Solvation Free Energy Results.[2]

System	Length of MD Run (ps)	$\Delta\Delta G^{Cal}$	$\Delta\Delta G^{Expt}$
CH_3COCH_3 — $HCOCH_3$[b]	RUN1 _510	−0.44 ± 0.22	−0.3
	RUN2 _510	−0.42 ± 0.21	
	RUN3 _510	−0.46 ± 0.23	
CH_3COCH_3 — $CH_3COCOOH$[b]	RUN1 _510	10.01 ± 0.72	
	RUN2 _510	10.10 ± 0.75	
	RUN3 _510	9.81 ± 0.73	
CH_3COCH_3 — CF_3COCH_3[b]	RUN1 _510	0.58 ± 0.25	
	RUN2 _510	0.62 ± 0.25	
	RUN3 _510	0.57 ± 0.24	
CH_3COCH_3 — CCl_3COCH_3[b]	RUN1 _510	3.79 ± 0.33	
	RUN2 _510	3.72 ± 0.32	
	RUN3 _510	3.76 ± 0.32	
CH_3COCH_3 — $CH_3COCOCH_3$[b]	RUN1 _510	3.78 ± 0.57	
	RUN2 _510	3.85 ± 0.55	
	RUN3 _510	3.81 ± 0.54	
CH_3COCH_3 — cyclopropanone[b]	RUN1 _510	−0.68 ± 0.59	
	RUN2 _510	−0.64 ± 0.57	
	RUN3 _510	−0.66 ± 0.62	
HCOH — $HC(OH)_2H$[b]	RUN1 _510	7.46 ± 0.67	
	RUN2 _510	7.35 ± 0.64	
	RUN3 _510	7.40 ± 0.62	

[a] Units are in kilocalories per mole.
[b] Each simulation started with different configurations.

Supplementary Material

A list of final atomic coordinates and CHELPG charges for the structures of compounds 1–9 following energy optimization at the 6-31G** basis set level is available (Table IV). In addition, nonstandard force field parameters, which are not available in the AMBER force field and Figures 6–9, are included. Available from authors upon request or see the footnote on the first page of this article.

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